

PRELIMINARY AMENDMENT
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transferase (cat) protein were measured in cell extracts. Levels of cat were expressed relative to uninduced levels set at 100%. Repression of >95% of the PKLlacOcat gene were observed in the presence of metal, and IPTG led to a derepression indicating that the observed repression was specific to the lac repressor. --

Page 10, lines 16-25, delete in their entirety, and insert therefor

-- Figure 5B shows, diagrammatically, the incorporation of lacO sequences in CMVTf to generate CMVlacOTF. The 18 base ideal lacO sequence was used to replace 18 bases in the CMV promoter between the TATAA (SEQ ID NO:4) box and transcription start point (tsp), and a dimer of that sequence was inserted into the restriction site *Pme* I, between the tsp and the ATG start codon. The replacement was achieved by incorporating the lacO sequence in an oligonucleotide then used as a primer in the Polymerase Chain Reaction (PCR). The insertion of the lacO dimer was done by ligation of a restriction enzyme fragment from the plasmid pOP (Hannan, G., et al 1994) into the *Pme* I site of PCMVtf. --

Page 10, lines 27-30, delete in their entirety, and insert therefor

-- Figure 5C provides a graph demonstrating the stable expression of CMVlacOTf in CHO/M(1)2lacIN. The plasmid pCMVlacOTf was transfected with CHO cells and stable transformants selected (labelled CHO/M(1)2lacIN cells). Cells from 2 clonal lines were grown for 12 hours in medium +/- metal and +/- IPTG, fresh medium added and the conditioned medium collected after another 24 hours. Tf levels were estimated (visually) on a Western blot and are expressed relative to levels in the absence of metal and IPTG

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which was set at 100%. Metal induced repression levels of >90% was observed in both clones and IPTG relieved most of that repression indicating its specificity. Levels of IPTG used (20mM) may have been sub-optimal thus explaining the incomplete derepression observed. --

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~~Page 11, lines 1-7, delete in their entirety.~~

~~Page 15, lines 8-20, delete in their entirety, and insert therefor~~

-- Example 1: The expression of transferrin or IGF-1 in CHO cells

Work over the last few years has been conducted in order to gain an understanding of the growth requirements of CHO-K1 in serum free medium (Crowley, J., 1989, Gray, P.P. et al., 1990 and Bridges, M., PhD Thesis). Long term growth from liquid nitrogen to large scale may be obtained with insulin or insulin-like growth factor (IGF), transferrin and fibronectin or laminin as the only exogenous proteins. Selenium also needs to be added as a trace element. A defined serum-free (SF) medium was developed referred to as UNSWSF+ITS. Slight changes in growth characteristics of the CHO-K1 cell line have occurred with different samples of CCL61 obtained over the years from American Type Culture Collection (ATCC). The current CHO K1 CCL61 stock obtained from ATCC in 1994 grows with a doubling time of around 17 hours in UNSWSF+ITS medium.

AS
~~Page 15, lines 23-28, delete in their entirety, and insert therefor~~

AE
-- The coding sequences for the IGF1 and transferring (Tf) genes, including the sequences for protein secretion, were isolated from a commercial human liver cDNA library (Clontech)

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Altered
using Polymerase Chain Reaction. Sequences 5' of the AUG start codon were modified to include an optimal translation initiation site (ACCATGA (SEQ ID NO:1) replacing AAGATGA (SEQ ID NO:2), Kozak, M. 1986). --

Page 18, lines 7-16, delete in their entirety, and insert therefor

-- Figure 4A shows that the presence of transferrin in defined medium, helped the long term growth and viability of CHO-K1 cells in culture. Figure 4B shows the final cell numbers and percentage viability of CHOSVLTf cells maintained from greater than 10 days in IS medium. It can be seen from Figure 4B that while the final cell densities for the three cell lines were similar, CHO-K1 growing on UNSWSF-ITS medium had a viability of 60%, similar to the CHOSVLTf cells expressing transferrin, while the viability of the CHO-K1 cells growing on the UNSWSF+IS medium was only 20%. This data shows the importance of having the CHO cells secreting transferrin, particularly for long term stable growth and viability. --

Page 19, lines 3-10, delete in their entirety and insert therefor

-- CHO cells were transfected using standard techniques with M(1)2lacIN and pSV2Neo. Cells were treated with 400 μ g/ml of G418 for 2 weeks and clonal cell lines resistant to G418 were selected. Clonal lines (designated CHO/R(5)4 and CHO/R(10)3) were obtained that produced low basal and high metal induced levels of repressor, as detected by Western blot. The repressor was shown to be biologically active by its ability to repress the

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gout
lacO-containing plasmid PGKlacOcat (Hannan, G., et al., 1994)
transiently introduced into the cells (Fig. 5A). --

Page 19, lines 13-20, delete in their entirety, and insert
therefor

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Page 19, lines 13-20, delete in their entirety, and insert
therefor
-- An ideal lac operator sequence (ATTGTGAGCGCTCACAAAT (SEQ ID NO:3)) based on the bacterial operator and rules for acceptable sites of insertion within a given promoter have been described (Hu & Davidson, 1987). The bacterial lac repressor has a high association constant for the ideal lac operator sequence which is a rare sequence with only three copies found in various mammalian genomes (Simons et al., 1984), thus offering good specificity of regulation of the target gene and minimal effect on the host genes (Simons et al., 1984). --

Page 19, lines 21-28; delete in their entirety, and insert
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Page 19, lines 21-28; delete in their entirety, and insert
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-- Lac operator sequences were inserted into the CMVTf gene to allow for repression by the lac repressor. One lac operator sequence (Fig. 5B) replaced promoter wild-type sequences between the TATAA (SEQ ID NO:4) box and the transcription start point and the other two were inserted between the transcription start point and the AUG start codon. Stable expression of this new construct (CMVlacOTf) in CHO cells already containing a stable inducible lac repressor gene (see example 3) was significantly shut down when repressor protein was present (i.e., the following metal induction) (Fig 5C). --

IN THE CLAIMS:

Please cancel Claims 1-6 and 17-21.